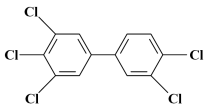
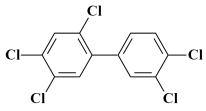
Binary Mixture of PCB126 and PCB118



3,3',4,4',5-pentachlorobiphenyl (PCB126)



2,3',4,4',5-pentachlorobiphenyl (PCB118)

PCB mixture study overview

- Not designed *a priori* as part of TEF Evaluation
- Originally a study of PCB118 alone
  - Doses: 0, 100, 220, 460, 1000, 4600 µg PCB118/kg
  - Based on TEF = 0.0001
- Rationale for study of PCB118
  - Highest exposure for mono-ortho class of PCBs
  - Has partial dioxin-like activity TEF = 0.0001
  - Mixed P450 inducer - Dioxin-like and Phenobarbital-like
    - Unclear if it should be included in TEF scheme

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PCB118 study aborted at 13 weeks

- Excessive toxicity in top dose groups
  - Premature deaths in 4600 µg/kg group
  - Body weight <90% of controls in 1000 ug/kg and 4600 ug/kg
    - More severe than TCDD at 14 weeks at 100 ng/kg
  - Near maximal induction of CYP1A1 at all doses
- Uncertainty in TEF of 0.0001 for PCB118
  - Range of RPFs in rats: 0.00001 - 0.0007
  - Not unexpected that potency could be higher
- Study redesigned and restarted
  - 500 µg/kg selected as highest dose
  - Predicted effects to be similar to TCDD/PCB126 study

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PCB 118 characterization

- Initial characterization of PCB 118; >98% pure
  - GC (system C)- three impurities; 0.2%, 0.8%, 0.5%
  - Impurities >0.1% but <1% are reported, but not identified as per NTP policy.
  - Not PCDDs or PCDFs
  - Synthesis route suggested impurities would not be PCBs of concern
- 13 week data prompted additional characterization
  - GC/MS (system E) : tetra-, hexa- and penta-CB
  - GC/MS (system F) characterization (% relative peak height)
    - 3,3',4,4'-TCB (PCB 77) (0.2%) TEF = 0.0001
    - 2,3,4,4',5,5'-HCB (PCB 167) (0.5%) TEF = 0.00001
    - 3,3',4,4',5-PCB (PCB 126) (0.8%) TEF = 0.1
- Further characterization of PCB126 since high potency PCB
  - GC (system D) using method of standard addition
  - PCB126 concentration = 0.622 +/- 0.061%

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Essentially a binary PCB "mixture"

Bulk "PCB 118" (µg/kg)	PCB 126 (ng TEQ/kg)	PCB 118 (ng TEQ/kg)	Group Name (ng TEQ/kg)
10	6.2	1.0	7
30	18.7	3.0	22
100	62.2	9.9	72
300	186.6	29.6	216
500	311.0	49.3	360

- PCB 126 and PCB 118: >99% of dioxin like activity
- Top two doses higher than other TEF studies
  - Max dose in other studies was 100 ng TEQ/kg

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Rationale for continuation of study

- PCB 126:153 studied to test for PCB interaction
  - Non ortho and di-ortho PCB interaction
- Humans co exposed to non-ortho and mono-ortho PCBs
  - Further evaluation of PCB interactions
  - PCB 126:118 ratio is environmentally relevant.

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Study Details-PCB126:PCB118

- Female Harlan Sprague-Dawley rat only
- Oral gavage: 5 days per week
- Vehicle: corn oil:acetone (99:1) - 2.5 ml/kg
- Doses: 0, 7, 22, 72, 216, 360 ng TEQ/kg
- Interim time points:
  - 14 and 31 weeks: All dose groups
  - 53 week: All except 360 ng/kg
- Stop-study 360 ng TEQ/kg (cease at 30 wks)

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Survival and body weight

- Effect on survival
  - Lower survival in 216 and 360 ng TEQ/kg groups and stop group
  - None in 216 and 360 ng TEQ/kg survived to end of study
- Decreased body weight gain
  - 72ng TEQ/kg and higher dose groups and “stop” group
  - Stop-group, near normal rate of gain after cessation of treatment
- Expected effects given higher doses
  - 100 ng TEQ/kg highest dose used in prior TEF studies
  - Not unexpected in top two dose groups given >100 ng TEQ/kg
  - Not predicted based on effects seen in aborted 13 week study

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Biochemical effects

- Increased cytochromes P450
  - Significantly increased at all doses examined at all time points
  - Liver CYP1A1 and CYP1A2
  - Liver CYP2B
  - Lung CYP1A2
- Alterations in thyroid hormones
  - Free and total T4 decreased
    - All time points, all doses 22 ng TEQ and higher
    - Total T4-all doses at 31 and 53 weeks
  - T3 decreased: only at 31 weeks only in 360 ng TEQ/kg
  - TSH increased: only at 31 weeks only in 216 and 360 ng TEQ/kg

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Hepatic toxicity: lesion spectrum

- Increasing dose and time
  - Increasing spectrum of effects
  - Increased severity
- Effects in 216 and 360 ng/kg
  - Shown in red
- 14 weeks
  - Hepatocyte hypertrophy
  - Pigmentation
  - Multinucleated hepatocytes
  - Fatty change, diffuse
  - "Toxic hepatopathy"
- 31 weeks
  - + Bile duct hyperplasia
  - + Centrilobular fibrosis
  - + Cholangiofibrosis
  - + Nodular hyperplasia
  - + Portal fibrosis
  - + Oval cell hyperplasia
  - + Focal cellular alteration
- 2 years
  - + Glandular structures
  - + Necrosis
  - + Bile duct cysts
  - + Centrilobular degeneration
  - + Metaplasia

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Liver: Lowest affected doses (ng/kg)

Endpoint	14wk	31wk	53wk	2-year
CYP1 P450 induction	7	7	7	
Rel liver weight increase	7	7	72	
Hepatocyte BrdU labeling	NS	216	72	
Hepatocyte hypertrophy	72	22	72	7
Toxic hepatopathy	216	216	72	7
Altered hepatic foci	--	216	NS	7
Bile duct hyperplasia	--	216	216	72
Oval cell hyperplasia	--	216	216	22
Nodular hyperplasia	--	360	216	22
Cholangiofibrosis	--	NS	216	72
Hepatocellular adenoma	--	--	--	216
Cholangiocarcinoma	--	--	--	22

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Liver: 2 year

	0	7	22	72	216	360	Stop
Animals per group	53	51	53	53	53	65	50
Adenoma	2* (5%)	1 (3%)	0 (0%)	4 (11%)	17* (56%)	5* (38%)	1 (5%)
Carcinoma	0	0	0	0	1	0	0
Cholangiocarcinoma[a]	0* (0%)	0 (0%)	5* (13%)	19* (48%)	28* (80%)	12* (69%)	19* (75%)
Hepatocholangioma[a]	0*	0	0	1	1	1	1
Cholangioma[a]	0	0	0	1	0	0	0

*P<0.05; [a]Historical control incidence 0/371; poly-3 incidence shown in parentheses

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Lung: 2 year

	0	7	22	72	216	360	Stop
Animals per group	53	51	53	53	53	66	50
Alveolar epithelium-metaplasia, bronchiolar	1*	14*	39*	46*	35*	8*	15*
Serosa, fibrosis	3*	0	0	1	16*	8*	1
Squamous metaplasia	0*	1	2	14*	16*	7*	8*
Cystic Keratinizing epithelioma (CKE) [a]	0* (0%)	0 (0%)	0 (0%)	20* (51%)	49* (98%)	41* (97%)	12* (43%)
Keratin Cysts	0	0	0	0	0	0	9*

*P<0.05 [a]Historical control incidence; 0/371

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Transplant studies

- Transplanted neoplasms from 216 ng TEQ/kg group
  - Moribund animals (days 672-709 on test)
  - >2mm fragments, 2-8 transplants per neoplasm
    - Cholangiocarcinoma - neoplasms from 7 rats - 33 transplants
    - CKE - neoplasms from 5 rats - 23 transplants
  - Athymic nude NCr mice or SCID mice
- Results
  - 3 months follow up
  - Regression (30-43%) or no fragment remaining (48-60%)
- Appendix to be added to TR
  - Data interpretation placed in perspective with other transplant studies

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Oral mucosa: 2 year

	0	7	22	72	216	360	Stop
Animals per group	53	51	53	53	53	66	50
Gingival squamous hyperplasia	11*	10	20*	24*	27*	18*	18*
Gingival squamous cell carcinoma[a]	1 (2%)	1 (3%)	2 (5%)	4 (11%)	0 (0%)	1 (10%)	1 (5%)

*P<0.05 [a] Historical control incidence; 3/211

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Pancreas: 2 year

	0	7	22	72	216	360	Stop
Animals per group	53	51	53	53	53	65	50
Acinar cytoplasmic vacuolization	0*	1	8*	39*	49*	43*	41*
Chronic active inflammation	1	5	4	3	2	6*	5*
Arterial chronic active inflammation	0*	2	2	21*	14*	4*	10*
Acinar atrophy	1*	5	3	5	9*	8*	8*
Acinar Adenoma/carcinoma	0	0	1	0	0	0	0

*P<0.05, Note asterisk for controls refers to trend test.

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Other organs: Non-neoplastic effects

- Thymic atrophy
- Thyroid follicular cell hypertrophy
- Adrenal gland - atrophy and cytoplasmic vacuolation
- Cardiomyopathy
- Nephropathy
- Spleen - lymphoid follicular atrophy
- Arterial chronic active inflammation
  - Mesentery, coronary, pancreatic
- Nasal Cavity
  - Respiratory epithelium - hyperplasia
  - Olfactory epithelium - metaplasia
- Forestomach - squamous hyperplasia
- Lymph node hemorrhage (several sites)

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Conclusions- PCB126:PCB118

- Clear evidence of carcinogenicity
- Based on
  - Cholangiocarcinoma of the liver
  - Hepatocellular neoplasms of the liver
    - Predominantly hepatocellular adenoma of the liver
    - Hepatocellular carcinoma of the liver
  - Cystic keratinizing epithelioma of the lung
- Also considered to be related to treatment
  - Gingival squamous cell carcinoma of the oral mucosa
- May have been related to treatment
  - Cholangioma of the liver
  - Hepatocholangioma of the liver

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